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Short Note

Repeated freezing impacts buoyancy and photosynthesis of a rafting kelp species

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Introduction

Antarctica was once considered biologically isolated, surrounded by oceanic barriers (Fraser *et al.* 2018). However, floating materials such as kelp rafts (Fraser *et al.* 2018, Avila *et al.* 2020), wood (Lewis *et al.* 2005) and plastics (Avila *et al.* 2020) are now known to cross these barriers and reach Antarctic shores. Such incursions might enable non-native species (either rafting species themselves or associated hitchhikers) to colonize Antarctica as the climate warms (Avila *et al.* 2020, Fraser *et al.* 2020), but whether these species will be able to survive and reproduce in the Antarctic is not yet known. Sea ice is a defining characteristic of Antarctic coastlines, and modelled trajectories of kelp rafts (Fraser *et al.* 2018) cross-referenced against sea-ice observations (Parkinson 2019) suggest that collisions between rafts and seasonal sea ice occur frequently (Fig. 1a); thus, rafts are expected to be entrained in, on or under sea ice and experience multiple freeze-and-thaw cycles on their journeys to Antarctica. In addition, kelp rafts that reach the Antarctic intertidal will experience temperatures well below 0°C if exposed to the air at low tide. Freezing can cause severe disruptive stress to seaweeds, and ice crystals growing in intercellular spaces can damage cell membranes and cause cell lysis (Eggert 2012). Such damage could affect the buoyancy of kelp tissue and decrease rafting ability. Although some non-native kelp rafts recovered from Antarctic shores appeared to still be reproductively viable, with mature gametes observed in reproductive tissue (Fraser *et al.* 2018), tissue damage caused by freezing could have widespread effects on the health, function and establishment success of a non-native species traversing the Southern Ocean.

We here used specimens of southern bull kelp (rimurapa; *Durvillaea antarctica*), a robust kelp that occurs throughout the high latitudes of the Southern Hemisphere and is one of the main algal species known to raft to Antarctica (Velásquez *et al.* 2020), to test the hypotheses that successive freezing-and-thawing events would 1) increase the amount of water absorbed, resulting in a decrease in positive buoyancy, and 2) decrease photosynthetic efficiency.

Methods

Durvillaea antarctica specimens were collected on three dates (23 November 2022, 6 December 2022 and 10 January 2023) from Brighton Beach, Aotearoa New Zealand (45.9468°S, 170.3348°E). Straps of distal blade tissue from ~10 individuals were cut transversely into pieces of ~50–70 mm length, and each piece was then divided into two, creating two short, roughly cylindrical 'twin' pieces from the same blade, each of which was then randomly allocated to either the control or the treatment group. Samples were stored separately in identical sealed, clear, plastic-lidded containers with 200 ml of sea water and incubated in the dark at 12°C for 1 day to allow samples to acclimate. Initial buoyancy, photosynthetic efficiency and cell damage were measured (see below). Treatment samples were then placed in a walk-in freezer with an air temperature of -20°C, and control samples were placed in a temperature-controlled room at 12°C. As the treatments were kept in a spatially separated unit from the controls and distal blade tissue from each individual was used to create multiple twinned tissue samples, this should be viewed as a pseudo-replicate design. All samples were kept in the dark during the experiments to control for light exposure.

Three treatment durations were tested, and in each, treatment samples were sequentially frozen three times for a set period of either 18, 24 or 48 h. Thirty control and thirty treatment samples were used for each

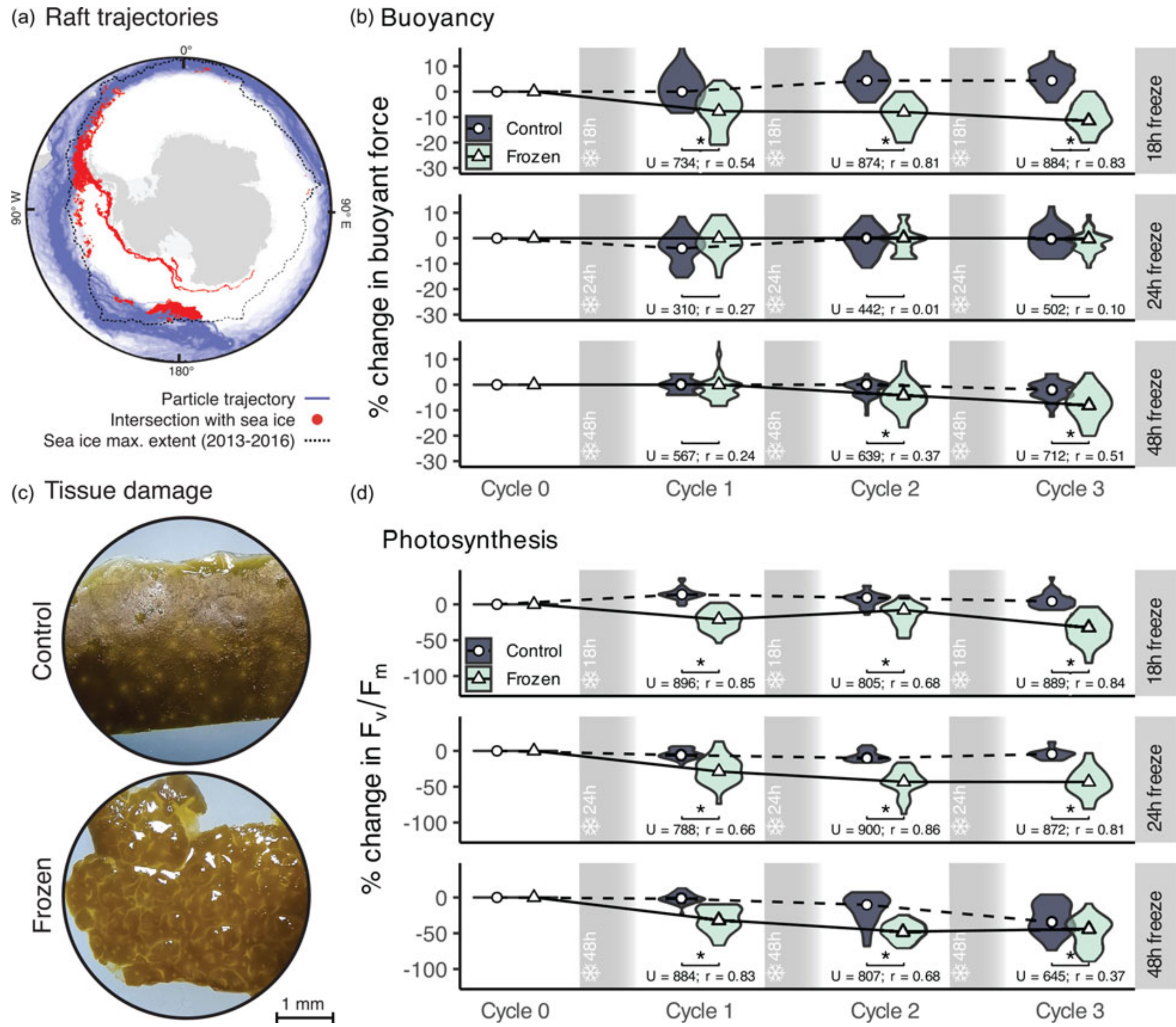

Figure 1. a. Rafting trajectories of kelp modelled against sea-ice observations to show interactions with sea ice around Antarctica. **b.** Tissue damage comparison of unfrozen (control) and frozen kelp external layers. **c.** Changes in buoyant force and **d.** photosynthetic efficiency (based on pulse amplitude-modulated fluorometer measurements) of frozen and unfrozen (control) kelp tissue samples undergoing three freeze cycles (indicated by grey shading) of either 18, 24 or 48 h in length. Results of Mann-Whitney U tests are indicated for each pairwise comparison (U = test statistic; r = effect size), with asterisks indicating statistically significant differences following Bonferroni correction. Central points represent median values, with full frequency distributions indicated by filled violins.

treatment. After each freeze period, a recovery period of 24 h occurred in the 12°C room for both the control and treatment groups. Buoyancy and photosynthetic efficiency were then measured on all 60 samples (30 control, 30 treatment) prior to the next freeze cycle. Thus, for example, 30 sub-replicate treatment samples were frozen together in the freezer for 18 h, thawed and allowed to recover for 24 h, tested, refrozen for another 18 h, thawed for 24 h, tested and frozen for another 18 h before a final 24 h thaw/recovery and test, whilst 30 sub-replicate control samples underwent the same tests at the same times but without freezing and in a separate room.

Buoyant force was approximated using Archimedes' principle; each sample was transferred to a measuring cylinder (precision ±0.5 ml) containing 100 ml of sea water and the volume of water displaced was recorded. For every measurement this was repeated twice, and the mean measurement was used in subsequent analyses. The photosynthetic efficiency of each kelp sample was measured based on the chlorophyll fluorescence of photosystem II via a pulse amplitude-modulated fluorometer (PAM; WALZ Diving PAM) after a 20 min period of complete darkness. The F values and M values were recorded, and the equation $F_v/F_m = (M - F)/M$ (where F_v is maximal variable fluorescence and F_m is

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maximal fluorescence yield) was used to calculate photosynthetic efficiency. PAM measurements were repeated on the alternate side of each kelp sample, and the mean F_v/F_m was used. For each kelp sample, the percentage changes, relative to initial measurements, in buoyant force and F_v/F_m over the course of the experiments were calculated. A generalized linear model (quasi-Poisson with a log-link function) was used to examine the effects of treatment type, treatment time, number of cycles and their interactions on either buoyancy or photosynthesis. The residuals of this model were checked to ensure that they met parametric assumptions. Pairwise Mann-Whitney U tests, with Bonferroni correction for multiple comparisons, were used to compare distributions of frozen and control samples at each stage of the experiment.

Results and discussion

Overall, freezing had a significant effect on the buoyancy and photosynthetic efficiency of southern bull kelp samples, with significant reductions of both observed across all but one of the frozen treatment groups (Fig. 1c, d & Table S1). Both 18 and 48 h freeze-cycle treatments showed a decrease in buoyancy, in line with previous research showing that damaged kelp tissue can lead to buoyancy reduction (Dufour 2011, Tala *et al.* 2019). Tissue damage can also lead to increased infection risk (Tala *et al.* 2013). Intriguingly, however, there was no significant decrease in buoyancy for the 24 h cycle despite significant declines in photosynthetic efficiency. Broadly, the first freezing had the greatest effect on the buoyancy of southern bull kelp, with subsequent freeze-thaw cycles only marginally decreasing buoyancy (Fig. 1c & Table S1). Our finding of a relatively small (4–12%) reduction in buoyancy after freezing could help explain how *D. antarctica* was found on Antarctic beaches after rafting for > 2 years, traversing > 20 000 km across the Southern Ocean (Fraser *et al.* 2022) and potentially encountering sea ice and freezing conditions on one or more occasions (Fig. 1a). Although bull kelp buoyancy is negatively affected by freezing, freezing alone is unlikely to cause enough damage to sink a raft, but it could be sufficient to alter raft geometry and associated properties such as drag and wind-driven drift; the latter has been shown to be critically important in enabling rafts to reach Antarctica (Fraser *et al.* 2018, 2022).

For kelp to establish, individuals would only need to survive long enough to be able to reproduce and reach close enough to the shore that sporelings can settle in the intertidal zone. *Durvillaea* eggs do not usually travel more than a few metres from the point of release (Dunmore 2006). Freezing had clear impacts on kelp condition and function, with signs of damage to the external layer of tissue (Fig. 1b) and decreased photosynthetic efficiency (Fig. 1d) observed across all treatment groups. Impacts on photosynthesis were most apparent after the first freeze and then began to plateau as control samples also started to exhibit declines in photosynthetic efficiency. Declines in the control group were most pronounced in the 48 h treatment (Table S1), which, including recovery periods, ran for 9 days, and mirrored the 2–3% daily declines described previously in *D. antarctica* rafts exposed to comparable ambient seawater temperatures (~12°C; Tala *et al.* 2019). The damage caused by freezing signals that, in spite of the preservative effects of low but above freezing temperatures at high latitudes (Tala *et al.* 2019), kelp rafts arriving on the Antarctic coastline after undergoing one or more freezing events will be in suboptimal condition and have a limited capacity to persist and grow. The degradation of external tissues observed here raises the question of whether freezing might also hinder reproductive viability, as gametes of this species are found in the surface cuticle where damage is most pronounced. Mature gametes have, however, been noted on *D. antarctica* rafts found on Antarctic coastlines (Fraser *et al.* 2022), indicating possible reproductive viability even if the adult rafting kelp itself is in poor condition. The initial process of freezing probably inflicts the most damage due to ice-crystal formation in kelp tissue. Therefore, repeated freezing events can be expected to cause increasing impacts on buoyancy and photosynthetic efficiency. Nevertheless, the effects of prolonged freezing events warrant further investigation.

We have demonstrated that freezing can impact the buoyancy and photosynthetic efficiency of a major rafting kelp species of the Southern Hemisphere, which has implications for the potential for rafts of non-native species to reach and possibly establish across Antarctic coastlines. Even small changes in buoyancy could alter raft geometry and influence raft trajectories (e.g. Stokes drift, which has been identified as an essential element in accurately predicting kelp raft trajectories (Fraser *et al.* 2018), will differentially affect rafts sitting higher or lower in the water), so understanding the effects of freezing and their interaction with the myriad other process that could degrade a kelp raft (Tala *et al.* 2013) will be important in modelling raft trajectories and forecasting incursions. Future research should assess how freezing affects the gametic cells and reproductive viability of *D. antarctica* and how declines in photosynthetic efficiency translate into longer-term effects on kelp survival and growth.

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Competing interests

The authors declare none.

Author contributions

SMS: conceptualization, methodology, formal analysis, investigation, writing - original draft, writing - review and editing. GAD: conceptualization, methodology, formal analysis, writing - review and editing, visualization, supervision. CIF: conceptualization, methodology, resources, writing - review and editing, supervision, funding acquisition.

Data availability

All data shown in this study are available in the Supplemental Material.

Supplemental material

A supplemental table and supplemental data file will be found at https://doi.org/10.1017/S0954102023000305.

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